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Fukutin mutations in congenital muscular dystrophies with defective glycosylation of dystroglycan in Korea

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ABSTRACT

This study was aimed to identify *Fukutin* (*FKTN*)-related congenital muscular dystrophies (CMD) with defective α-dystroglycan glycosylation in Korea and to discuss their genotype–phenotype spectrum focusing on detailed brain magnetic resonance imaging (MRI) findings. *FKTN* mutations were found in nine of the 12 CMD patients with defective α-dystroglycan glycosylation patients (75%). Two patients were homozygous for the Japanese founder retrotransposal insertion mutation. Seven patients were heterozygous for the retrotransposal insertion mutation, five of whom carried a novel intronic mutation that activates a pseudoexon between exons 5 and 6 (c.647+2084G>T). Compared with individuals that were homozygous for the retrotransposal insertion mutation, the seven heterozygotes for the retrotransposal insertion mutation, including five patients with the novel pseudoexon mutation, exhibited a more severe clinical phenotype in terms of motor abilities and more extensive brain MRI abnormalities (i.e., a wider distribution of cortical malformation and pons and cerebellar hypoplasia). *FKTN* mutations are the most common genetic cause of CMD with defective α-dystroglycan glycosylation in Korea. Compound heterozygosity of the retrotransposal insertion and the novel pseudoexon mutation is the most prevalent genotype in Korea and is associated with a more severe clinical and radiological phenotype compared with homozygosity for the retrotransposal insertion mutation.

1. Introduction

The identification of defective glycosylation of α-dystroglycan (α-DG) in muscle and brain as an important cause of progressive muscle degeneration and abnormal neuronal migration in the brain [1,2] led to the collective classification of a heterogeneous group of congenital muscular dystrophies (CMDs) as CMD with defective glycosylation of α-DG. To date, six genes associated with these disorders have been identified: *Fukutin* (*FKTN*; OMIM 607440), *Protein-O-mannose 1,2-N-acetylglucosaminyltransferase 1* (*POMGnT1*; OMIM 606822), *Fukutin-related protein* (*FKRP*; OMIM 606596), *Protein-O-mannosyl transferase 1* (*POMT1*; OMIM 607423), *LARGE* (OMIM 603590), and *Protein-O-mannosyl transferase 2* (*POMT2*; OMIM 607439) [3–8]. Clinically, Fukuyama CMD (FCMD), muscle–eye–brain disease (MEB), and Walker–Warburg syndrome (WWS) are among the most frequently reported phenotypes of CMD with defective glycosylation of α-DG. Although the two most recent studies using large cohorts of subjects demonstrated overlapping spectra among these six genes and clinical phenotypes [9,10], each syndrome was described originally in distinct ethnic groups as having mutations in specific genes. MEB is most prevalent in the Finnish population and is associated with the high prevalence of a founder splice-site mutation in *POMGnT1* [11]. The founder retrotransposal (RT) insertion mutation in the 3′-untranslated region (UTR) of *FKTN* is responsible for the high incidence of FCMD in the Japanese population [3]. Therefore, although the genotype–phenotype spectrum of these diseases is heterogeneous, the prevalence of mutations among the listed genes may vary according to the different ethnic groups, as observed for *POMGnT1* in Finland and *FKTN* in Japan.

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In 2009, one Korean and one Chinese CMD patient were reported as having mutations in *FKTN* [12,13]. Interestingly, the RT insertion mutation, which had not been reported outside of Japan, was identified in either the heterozygous or the homozygous state in these patients. Based on the ethnic similarity between the Japanese and Korean populations, as well as on these recent reports, we hypothesized that mutation in *FKTN* is also prevalent in Korea. Thus, we performed a mutational analysis of *FKTN* in Korean patients with CMD with defective glycosylation of α-DG. We also discussed their genotype–phenotype spectrum, focusing on detailed brain magnetic resonance imaging (MRI) findings.

2. Subjects and methods

2.1. Patients and clinical data

The Institutional Review Board of the Seoul National University Hospital approved the study protocol. The study subjects consisted of 12 cases diagnosed and followed at the Seoul National University Children's Hospital from 2000 to 2008. The inclusion criteria were presence of hypoglycosylation of -DG on the sarcolemma of skeletal muscle sections, as assessed using immunohistochemistry [14], and a clinical diagnosis of CMD characterized by early onset muscle weakness or hypotonia at <2 years of age, delayed developmental milestones, and increased serum creatine kinase. Muscle biopsy specimens were unavailable for one female case; nevertheless, this patient was included in the study because she exhibited characteristic clinical findings that were highly suggestive of CMD with defective glycosylation of -DG, i.e., early onset muscle weakness, elevated serum creatine kinase (CK) levels, brain involvement, and a family history of the disease. Clinical and laboratory data, including ophthalmological findings, CK levels, presence of epilepsy or seizure, motor and language development, and family history, were reviewed. Clinical severity was classified as either severe or mild. A diagnosis of severe phenotype was attributed to patients who could not sit without support up to 5 years of age or who could not hold their head upright up to 2 years of age. A mild phenotype was attributed to patients who could sit without support or perform more complex motor tasks, regardless of age. One Korean patient [12] previously identified as having a homozygous RT insertion mutation in *FKTN* was included in the analysis of the genotype–phenotype spectrum. This patient was designated as Case 13.

2.2. FKTN mutational analysis

2.2.1. Three-primer PCR and direct sequencing of FKTN

After obtaining informed consent, genomic DNA was extracted from either peripheral blood leukocytes or muscle samples using a Wizard Genomic DNA Purification kit, according to the manufacturer's instructions (Promega, Madison, WI). A three-primer PCR using LAT7ura, LAT7–2, and ins385–359 was performed, as described previously [15]. Direct sequencing of all coding exons and flanking intronic sequences of the *FKTN* gene was performed using primer pairs designed by the authors (available upon request). PCR was performed in a thermal cycler (Model 9700; Applied Biosystems, Foster City, CA) and cycle sequencing was performed on an ABI Prism 3100xl Genetic Analyzer using the BigDye Terminator Sequencing Ready Reaction Kit (Applied Biosystems). Sequence variations were analyzed via comparison with the wild-type sequence (GenBank Accession No. NM_006731).

2.2.2. RT-PCR-based sequencing

Total muscle RNA was extracted from frozen muscle biopsy specimens using Trizol® (Invitrogen, CA, USA) and cDNA was synthesized from total muscle RNA using an iScript™ cDNA synthesis kit (Bio-Rad, CA), according to the manufacturer's instructions. To assess the presence of splicing aberrations in the FKTN transcripts, RT-PCR-based sequencing was performed using five pairs of primers (RT-F1, CAGCCTGCTGTTGAGTGAGA; RT-R1, GCTATCCGAAACCAGCCTTC; RT-F2, TGGCTCTACTTCACAATGCAA; RT-R2, TGCTCGAGCTTCTTTATACCTACA; RT-F3, GACAGGCCAGA GTTACAGCA; RT-R3, CCATTCCACATGTGATCAGTTT; RT-F4, AGCA TTTCAGGATGCAGGAC; RT-R4, TGGTTCCCACTTATGTTTGACA; RT-F5, TGGAATCTGGCCTATTTCTGA; RT-R5, GCACTAACATACCAGCTTA AATGC). PCR products were separated either on agarose gels or on ScreenTape using a Lab901 system (Lab901 Ltd., Loanhead, UK). Any PCR fragments of aberrant size were sequenced directly or after a cloning procedure using the TOPO TA Cloning Kit (Invitrogen).

2.3. Brain MRI

All MRI data were reviewed by two radiologists (I.O.K. and Y.J.L.) who were blinded to the patients' clinical history and to the results of the mutational analysis. Four categories of abnormalities were reviewed: cortical malformation (polymicrogyria and cobblestone-type lissencephaly), infratentorial cerebellar abnormalities (pons hypoplasia, cerebellar hypoplasia, and cerebellar cysts), white-matter changes, and ventricular dilatation.

2.4. Muscle immunohistochemistry

Muscle samples that were collected with informed consent for diagnostic purposes were frozen in isopentane chilled with liquid nitrogen. Commercially available monoclonal antibodies to α-DG (VIA4-1; Upstate Biotechnology, Lake Placid, NY) and to β-dystroglycan (43DAG1/8D5; Novocastra, Newcastle upon Tyne, UK) were used for immunohistochemical staining.

3. Results

3.1. Clinical characteristics

The detailed clinical information of the 12 cases (Cases 1–12) that were tested for *FKTN* mutation is presented in Table 1. Among the six cases that were followed after age 5, three cases could not sit without support. Among the six cases that were not followed-up to age 5, four cases could not hold their head upright, even after 2 years of age. Seizures were present in four cases. Eye abnormalities were detected in six cases: myopia in four patients, strabismus in three patients, and cataract in two cases. Cases 8 and 12 had elder siblings with a similar clinical history that was suggestive of CMD; however, there was limited additional information, as the elder brother of Case 8 died at 7 years of age without a confirmation of the diagnosis and the parents of Case 12 refused the evaluation of the elder sister of this patient. The two maternal aunts of Case 2 seemed to suffer from a muscle disease with late onset, as assessed using history alone.

3.2. FKTN mutational analysis

3.2.1. Three-primer PCR and direct sequencing of FKTN

The three-primer PCR revealed that two cases (Cases 1 and 2) were homozygous for the RT insertion mutation and seven cases (Cases 3–9) were heterozygous for the RT insertion mutation (Fig. 1A). The RT insertion mutation was present in nine of the 12 cases (75%) in at least one allele. Direct sequencing of *FTKN* exons and their boundaries was performed in 10 cases (i.e., seven cases that were heterozygous for the RT insertion mutation and